ON THE RELATION BETWEEN TENSION AND ATP IN CROSS-STRIATED MUSCLE

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According to the theory outlined in the preceding paper, the contractile matter of muscle is built of functional units containing myosin, actin, and ATP. Since muscle contains no free ATP, it can be expected that if the ATP concentration of muscle decreases, the number of contractile units decreases proportionately. The ATP concentration of muscle decreases after the death of the animal (Th. Erdös, 1943). The object of the present research was to see whether the ATP content and the tension developed by muscle decrease proportionately. Such a parallelism would support the theory outlined, while a lack of parallelism would plead against it. For this reason, we measured, simultaneously, the tension developed by the muscle and the ATP concentration at various intervals after the death of the animal.

The material used was the musculus psoas of the rabbit. At various intervals after the death of the animal, strips of this muscle were cut out and frozen at once. The tension developed on thawing was measured.

The methods hitherto used for the estimation of ATP were found to be unsatisfactory for the following reason: we possess no direct method for the estimation of ATP. When this substance has to be estimated, extracts of the tissue are subjected to limited acid-hydrolysis, and the quantity of ATP is calculated from the quantity of labile phosphate liberated. Muscle contains *in vivo* a not inconsiderable amount of free phosphate. As the ATP is gradually decomposed *post mortem*, the amount of hydrolyzable phosphate decreases while the amount of free phosphate increases, and thus a slight error in the phosphate estimation makes the results of the ATP estimation doubtful. A new method of phosphate estimation had to be constructed in which the free phosphate did not interfere with the estimation of the ATP.

In the first part of this paper this method will be described. In the second part, the results obtained by this method will be given.

Method of Phosphate Estimation

The method is based on the ready solubility of phosphomolybdic acid in iso-butyl alcohol, described by Berenblum and Chain (1938), and on the yellow color with which the acid dissolves in this reagent. The muscle was extracted with trichloracetic acid. Ammonium molybdate was added to the solution. The free phosphate present combined with the molybdate and was shaken out with a mixture

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of iso-butyl alcohol and ethyl ether. Then the fluid was hydrolyzed and the free phosphate shaken out with iso-butyl alcohol and estimated colorimetrically.

After the trichloracetic acid extract of the muscle is shaken out with butyl alcohol ether, it still contains a small quantity of phosphate. This quantity can be estimated and taken into account. If the extract is shaken out a second time with alcohol ether, no phosphate is left, and no correction has to be made on the final readings. In the present paper the former method was used.[3]

Muscle extract contains substances which, after boiling with HCl, yield products which interfere with the development of the yellow color. These substances are eliminated by the alcohol ether, since they are of lipoidic nature.

The detailed description of the procedure is as follows: the rabbit (2–3 kg.) was decapitated, eviscerated, the side walls of the abdomen cut off and the psoas exposed. Two thin strips of the psoas were taken out, provided with ligatures, fixed and frozen with dry ice at their resting length, as described in the preceding paper. These strips were used for estimating the maximum tension developed by the muscle on thawing at 15° C. Simultaneously, a somewhat thicker strip of about one gram weight was cut out from the same region, weighed and frozen. This strip served as material for the ATP estimation. The remainder of the muscle, left *in situ,* was covered with cotton wool wetted with Ringer. The procedure was repeated once every hour. First the right and then the left psoas was used. Such samples were taken until the muscle showed no elasticity and no contractility after thawing. One hour later a last sample was taken.

Extraction: 25 ml. of 10 per cent trichloracetic acid was pipetted into a mortar which was pre-cooled to − 20° C. The fluid solidified to a brei. The muscle, after having been weighed, was placed into the brei in frozen condition and ground to a fine suspension. On thawing, the suspension was transferred into a centrifuge tube and spun. The clear fluid was poured into a 50 ml. graduated measuring cylinder provided with a ground glass stopper. The volume was noted; then for every 10 ml., 1 ml. of 10 per cent ammonium molybdate solution was added and the fluid mixed. Then 1 ml. of iso-butyl alcohol was added for every 4 ml. of the fluid, and 4 ml. of ether added for every ml. of butyl alcohol used. The fluid was strongly shaken for twenty-five seconds and allowed to separate. If there was no ready separation of the two phases, the fluid was centrifuged. Then the ether butyl alcohol mixture was sucked off through a capillary glass tube. A few ml. of ether were added without shaking in order to wash off the remaining alcohol ether. The volume of the fluid was noted. If, after the shaking with alcohol ether, a heavy precipitate was formed, this was separated by centrifugation. The fluid was divided into samples, each of which corresponded to 100 mg. of muscle, and pipetted into test tubes. Out of nine samples four were put aside. To five samples, 1/10 parts of concentrated HCl (approximately 10 N) was added and the tubes placed into the boiling water-bath for seven minutes and then rapidly cooled. To the unboiled samples, the same amount of HCl was added. To all tubes one drop of 0.1 per cent potassium permanganate was added which stained the fluid a rose color. This color persisted for about half a minute. This was done in order to oxidize any reducing agent present which would reduce the phosphomolybdate. Then 10 per cent ammonium

[3] If for any reason the quantity of free phosphate present in the muscle extract had to be known, this could be estimated colorimetrically in the combined alcohol ether extracts.

molybdate was added to the unboiled tubes, and 5 ml. iso-butyl alcohol to all samples. The butyl alcohol used here was shaken out previously with water. (This is necessary in order to prevent the butyl alcohol from taking up water later.) The fluid was shaken strongly for five seconds, the opening of the tube being closed by the thumb covered by a rubber glove. After the two phases separated, the watery phase at the bottom was sucked off by means of a thin glass tube, connected to the vacuum by a thin rubber tube which was pinched tight while the tip of the tube was passing the alcohol. Then the alcohol was poured over into the colorimeter tubes which were marked at their 5 ml. volume. Usually the volume of butyl alcohol is less than 5 ml. It was filled up to 5 ml. with butyl alcohol which was used to rinse the tubes that contained the extract previously. Then to every tube 1 ml. of ethyl alcohol was added and the color estimated in the Klett-Summerson colorimeter with the S 42 blue light filter (400–460 mμ).

As a standard, a solution of KH_2PO_4 was used, containing 0.01 mg. per ml. Samples of 1, 2 and 3 ml. of this fluid were filled up with water to 4 ml., 0.5 ml. cc. HCl and 0.5 ml. of 10 per cent molybdate were added; then the fluid was shaken out with 5 ml. butyl alcohol which was treated as described above.

Experimental Results

Before embarking on the problem proper, a few minor points had to be cleared up. First, is the method of P estimation reliable, and is the distribution of ATP in the psoas homogeneous?

A rabbit was killed and six samples of 1 g. were taken from different parts of the two psoas muscles. In Table I the actual colorimeter readings are reproduced. The six upper columns related to the unhydrolyzed extract are thus the zero values. The corresponding readings of the hydrolyzed samples are reproduced in the lower columns.

As can be seen, the readings are very uniform. The one value in the fourth column, marked with an asterisk, is evidently due to some rough mistake and has to be discounted. The other single values do not differ from the average by more than five per cent. The average of the 0 value was substracted from the average of the hydrolyzed product. From this the ATP was calculated. The standard with

Table I

	40	38	30	29	24	29
	39	35	30	69*	24	28
	39	37	30	29	24	28
	202	194	190	204	198	204
	200	189	200	200	194	202
	204	194	198	206	196	202
	190	196	189	200	200	198
	196	199	187	204	208	194
	195	186	198	214	195	195
Average	198	194	194	205	198	199
ATP	3.55	3.50	3.47	3.70	3.68	3.60 mg. per gm.

0.02 mg. phosphate gave a reading of 80. The quantity of P found was multiplied by 8.4 to give the ATP, which is noted in the last horizontal line. This shows the ATP content of the psoas to be very uniform, 3.6 mg. ATP per gm.

According to the literature, muscle contains 2–2.5 mg. ATP per gm., thus considerably less than psoas. This difference is probably due to the shielded position of the psoas and the consequent poverty of connective material. In order to elucidate this point, samples of different muscles of a freshly killed rabbit were taken and subjected to analysis. Results are reproduced in Table II.

TABLE II

Psoas	3.55 (mg. ATP per gm.)
Deep muscles of the back	3.04
Big adductor muscle	2.56
Musculus gracilis	2.10
Superficial muscle of the back	2.10
Smaller muscles from the gluteal region	1.96

These values show that the more superficial the position and the richer the connective tissue, the lower the ATP content. The muscles of the whole animal would give an average of about 2.5 mg. ATP per gm.

The third question which had to be cleared up was whether the ATP content of the psoas decreases uniformly in all its parts after the death of the animal. Preliminary experiments have shown that the rate of disappearance of ATP *post mortem* depends on the temperature and the oxygen supply. If the muscle is cut into thin strips which are exposed to air, the disappearance becomes much slower. While the ATP in the muscle left *in situ* may disappear within three to four hours, muscle strips exposed to air may contain ATP and thus remain contractile at room temperature even twenty-four hours after the death of the animal. Experiment also showed that in the muscle left *in situ*, the ATP disappeared faster in the deeper-lying dorsal than in the superficial ventral part.

Experiment: The rabbit was killed, the psoas exposed as usual and covered with wet cotton wool. Three hours later, five strips, weighing approximately 1 gm., were cut out and analyzed for ATP. One of the strips was taken from the lateral edge of the muscle, two from the ventral surface, two from the deeper-lying dorsal surface. ATP (mg.) per gm:

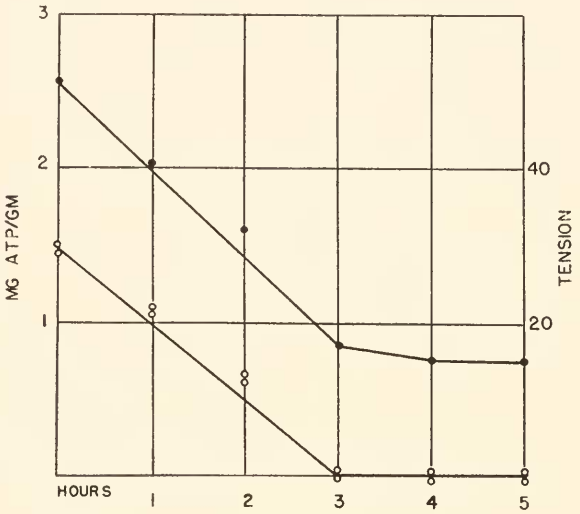
edge:	2.53	
ventral:	2.77	2.77
dorsal:	1.80	1.95

If the ATP content of muscle and its tension are to be measured simultaneously, it is essential that strips from the same region be used for both measurements. Even with this precaution, considerable scattering of results can be expected.

The relation between ATP content and tension was studied in eight experiments. The following example may be cited (Fig. 1): Samples of muscle were taken every hour after the death of the animal. The ATP content in milligrams per gram of muscle is marked in the curve by points. They relate to the left ordinate.

The tension developed is marked with circles and refers to the right-hand side ordinate. (The scale of this ordinate is arbitrary and is chosen in such a way that the numbers, if multiplied by 100, give the total working capacity in calories calculated for 35,000 gm. myosin by the formula: ⅓ tension × length × 0.000023.)

As the curve shows, tension and ATP content run parallel. At the end of the third hour the muscle develops no more tension and does not contract on thawing, and is found to be completely inelastic. At this point, the ATP curve shows a break and becomes roughly parallel to the abscissa.



As the curve shows, the muscle at this point still contains a not inconsiderable amount of labile phosphate. Whether this hydrolyzable ATP is derived from ATP or some other source (ADP?) cannot be stated at present. If this hydrolyzable P is derived from ATP, this ATP must be in some way different from the rest, because it is no longer split by the muscle (or is split only exceedingly slowly) and has no influence on contractility and elasticity. This "residual" hydrolyzable phosphate was found in approximately the same proportion in all experiments.

The second point, equally borne out by the other experiments, is that the decrease of ATP concentration is linear: the rate of its disappearance is independent of its concentration. The most likely interpretation of this rather unexpected fact is that the splitting of ATP depends on some change in the contractile matter. As has been shown by A. Biro and A. E. Szent-Györgyi (unpublished), myosin is enzymatically active in its contracted condition only.

In two out of the eight experiments, the ATP concentration did not fall at all during the first hour after death. This can be explained by the presence of creatine-phosphate which rephosphorylates the ADP formed.

All experiments gave similar results. In most of them the scattering was stronger than in the quoted example. Nevertheless, all experiments bore out the close parallelism between tension developed and the quantity of ATP present.

SUMMARY

A new colorimetric method of ATP estimation is described. In the psoas of the rabbit the *post mortem* decomposition of ATP and the loss of contractility are parallel.

LITERATURE CITED

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